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Micro-Anatomy Imager

S. G. Demos

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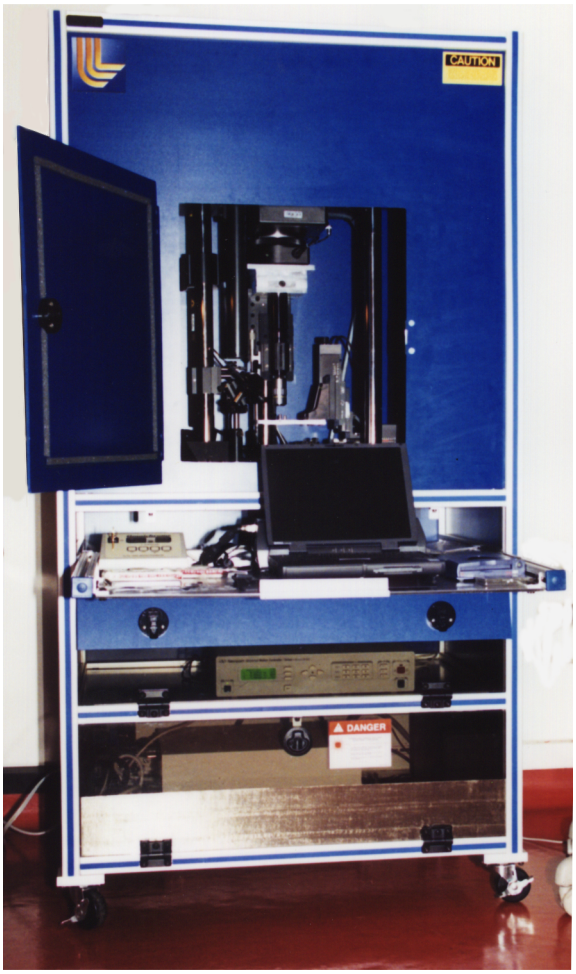
Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

2013 R&D 100 Awards Entry Form
Lawrence Livermore National Laboratory

Micro-Anatomy Imager

Real-time imaging of tissue cells structure and organization without prior tissue preparation for faster disease diagnosis.



Auspices Statement
This work performed under the auspices of the U. S. Department of Energy (DOE) by LLNL under Contract DE-AC52-07NA27344.

1. ORGANIZATION CONTACT INFORMATION

A. Submitter Information

Organization Name: Lawrence Livermore National Laboratory
Contact Name: Stavros G. Demos
Address: 7000 East Ave.
City/State: Livermore, CA
Zip/Postal Code: 94550-9234
Country: USA
Phone: 925-423-3388
Fax: 925-422-5718
Email: demos1@llnl.gov
Web URL:

Indicate relationship to product being submitted:
__ Public Relations or Marketing Agency
X Product Developer
__ Other (explain) _____

(Provide marketing and media contacts in Appendix B)

B. Developer Organization Information

List *all organizations* that participated in or contributed to the R&D process for this product. Copy the template to add additional organizations.

If the developer was listed in question 1A, the information must also be listed in 1B. Any organizations excluded from question 1B will not be publicized if the entry wins.

Organization Name: Lawrence Livermore National Laboratory

What role did this organization play in development of technology?
X Principal developer organization (All or majority of development responsibility)
__Co-developer organization (Equal share of development responsibility with other organizations)
__Supporting developer organization (Contributed less than 50% of development responsibility)

Address: 7000 East Ave.
City/State: Livermore, CA
Zip/Postal Code: 94550-9234
Country: USA
Phone: 925-423-3388
Fax: 925-422-5718
Email: demos1@llnl.gov

Web URL: www.llnl.gov

Provide details of development role: LLNL led the development of the instrumentation and associated technology.

Organization Name: University of California, Davis

What role did this organization play in development of technology?
_ Principal developer organization (All or majority of development responsibility)
_ Co-developer organization (Equal share of development responsibility with other organizations)
X Supporting developer organization (Contributed less than 50% of development responsibility)

Provide details of development role: Tested instrumentation in clinically relevant conditions.

Address:	1 Shields Ave
City:	Davis
State:	California
Zip/Postal:	95616
Country:	USA
Web URL:	www.ucdavis.edu
Contact Name:	Shiro Urayama, M.D.
Phone:	916-734-3759
Fax:	916-734-7908
Email:	surayama@ucdavis.edu

C. Principal/primary investigator, developer, inventor, or team leader

Principal Developer Name:	Stavros G. Demos
Title:	Scientist
Organization:	LLNL
Address:	7000 East Avenue
City/State:	Livermore, California
Zip/Postal:	94550
Country:	USA
Phone:	925-423-3388
Fax:	925-422-5718
Email:	demos1@llnl.gov

Developer, inventor name:	Shiro Urayama, M.D.
Title:	Professor

Organization:	University of California, Davis
Address:	4150 V Street, PSSB Suite 3500
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA.
Phone:	916-734-3759
Fax:	916-734-7908
Email:	surayama@ucdavis.edu

Developer, inventor name:	Bevin Lin
Title:	Graduate Student Researcher
Organization:	NSF Center for Biophotonics Science & Technology, University of California, Davis
Address:	2700 Stockton Blvd., Suite 1400
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA
Phone:	916-734-8600
Fax:	916-703-5012
Email:	belin@ucdavis.edu

Developer, inventor name:	Ramez Saroufeem, M.D.
Title:	Associate Professor
Organization:	University of California, Davis
Address:	4400 V Street, PATH Building
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA.
Phone:	916-734-2529
Fax:	916-734-2560
Email:	ramez.saroufeem@ucdmc.ucdavis.edu

2. PRODUCT INFORMATION

Micro-Anatomy Imager

B. Generic description of product (spectrometer, battery, chemical, etc.)

Microscope for medical diagnosis and research.

C. Product photo

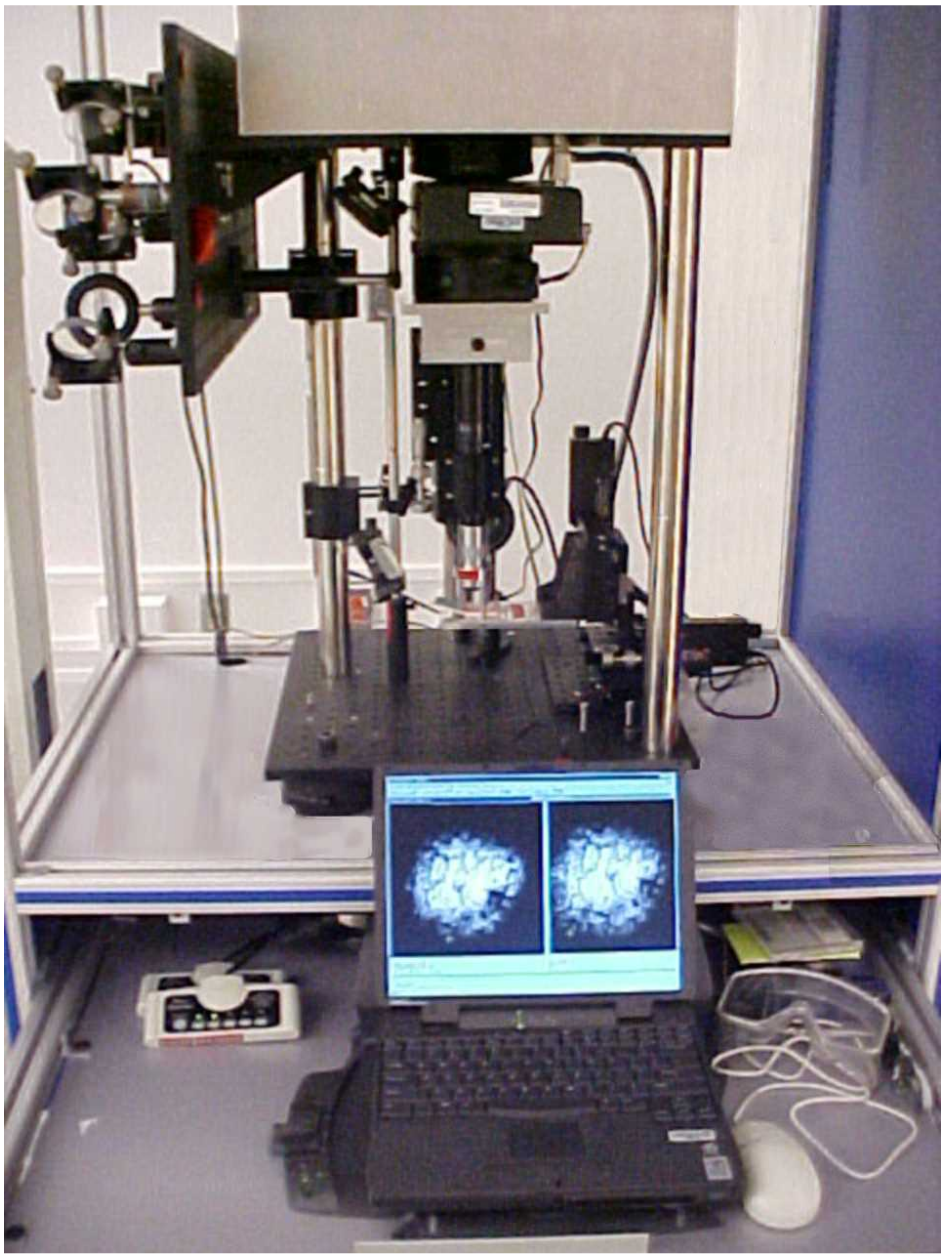


Figure 1. The Micro-Anatomy Imager provides detailed images of intact tissue cells without requiring any tissue preparation to permit rapid medical diagnosis. Above, the instrument's microscope and two images of tissue cells it acquired.

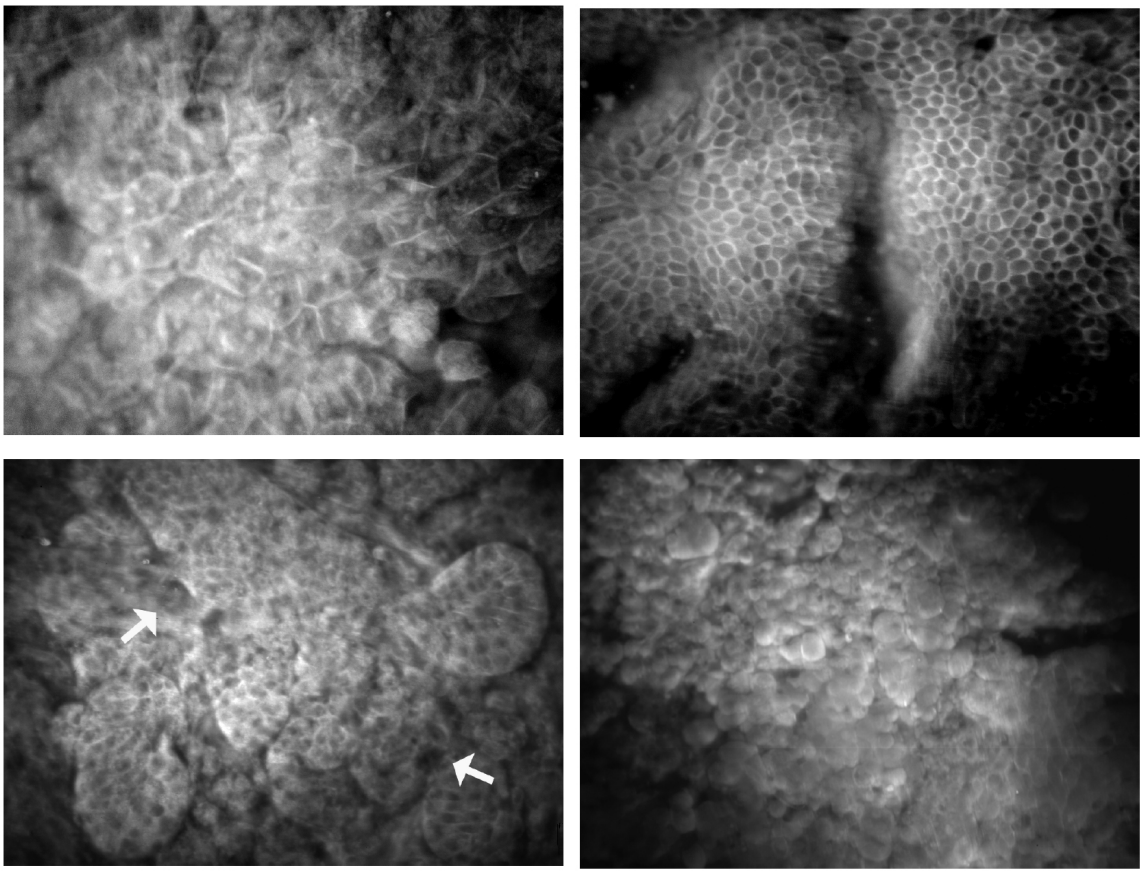


Figure 2. Images of the esophagus taken with a prototype Micro-Anatomy Imager showing (top) healthy tissue cell structure and (bottom left) Barrett's esophagus, a premalignant condition, and (bottom right) high-grade dysplasia, marked by cauliflower-like malignant projections.

3. EXECUTIVE SUMMARY

The Micro-Anatomy Imager provides noncontact visualization of tissue cell structure and organization to detect abnormal changes associated with the presence of disease such as cancer. For the first time, visualization of the structure and function of live cells at the tissue level is possible (Figures 1 and 2). Images can be acquired without any need for prior preparation of the tissue or use of contrast agents. The technology can be used to rapidly evaluate excised tissue specimens of patients during surgery to determine if, for example, all cancer has been removed. The instrument addresses current limitations in histological examination of tissue specimens obtained during surgery to help advance patient care and reduce healthcare costs. The technology is simple and inexpensive. This technology can also be used in experimental in vivo animal research, to enhance education in biology and, in future implementation, as an imaging modality that can visualize cancer lesions in real time to guide surgery.

4. INTRODUCTION DATE

November 2012.

5. PREVIOUS R&D 100 ENTRY

No.

6. PRICE

The price of the Micro-Anatomy Imager ranges from $10,000 to $150,000, depending on features.

7. PATENTS

1. Demos Stavros G., *Hyperspectral microscope for in vivo imaging of microstructures and cells in tissues*, US7945077B2, 2011-05-17
2. Demos Stavros G, Urayama Shiro, Lin Bevin, Saroufeem Ramez Moussa Ghobrial, *In vivo spectral micro-imaging of tissue*, US8320650, 2012-11-27.

8. PRODUCT DESCRIPTION

A. What does it do?

The Micro-Anatomy Imager enables real-time acquisition and display of digital images depicting the microstructure of the surface layer of intact tissues. The tissue surface may be either the surface of an organ or the exposed surface via surgical dissection. By enabling the speedy visualization of critical microscopic changes associated with the presence of disease, diagnostic information is provided in real time for faster diagnosis by the surgeon or a pathologist located either nearby or at a remote site. A key feature is that the instrument does not require any tissue preparation or direct contact with the tissue. Image acquisition is rapid: the entire surface of large specimens can be imaged with sub-micron resolution in less than a minute. The images are formed using native tissue biomolecules that produce a fluorescent signal under ultraviolet excitation. The Micro-Anatomy Imager can also be operated using contrast agents to easily provide enhanced visualization of specific components of cells.

B. Describe the principal applications of this product.

Surgical tissue margins (outer tissue edges), lymph nodes, and other tissue samples often need to be assessed during surgery, especially with the trend towards “organ

sparing" procedures that attempt to remove as little tissue as possible while not cutting across the tumor or leaving tumor behind. Diagnosing a specimen during an operation can be slow and require tissue preparation that prolongs and complicates a complete assessment. Currently, a tissue sample from the patient is sent to the frozen section lab for evaluation. Processing involves sub-sampling, orientation, tissue freezing, sectioning, slide preparation, staining, and evaluation under a light microscope by a pathologist. If a margin shows evidence of tumor, additional tissue must be taken until a clear margin is achieved. It can typically take 20 minutes for the examination of a single small piece of tissue and correspondingly longer for each additional sample. Furthermore, this current method of margin assessment often suffers from sampling error due to the inability to get the entire margin on a microscope slide. These sampling problems may require additional surgery or additional treatments (such as radiotherapy or chemotherapy). In addition, damage to the frozen specimens from fixation, infiltration, embedding, and sectioning may complicate the pathologist's interpretations.

The Micro-Anatomy Imager addresses these recurrent problems. Specimens are imaged rapidly and a digital image capturing the microanatomy of the surface layer of the specimen is obtained within about 1 minute, depending on the size of the specimen. The image permits detailed visualization of critical structures such as the presence of tumor at the specimen margins. The microscope system is optimally located in a room adjacent to the operating room. Physicians performing the operation can immediately examine the image of the specimen, or the image can be sent digitally via the Internet for examination by a pathologist located in a distant facility.

C. How will this product benefit the market that it serves?

The Micro-Anatomy Imager and associated technology permits examination of a specimen at least an order of magnitude faster than current rapid tissue processing via frozen section, which takes about 20 minutes. This advance will lead to faster diagnoses during an operation, which in turn will reduce the duration of the surgery and consequently the risk for the patient as well as the cost.

With the Micro-Anatomy Imager, the tissue specimen remains intact, unaffected in any way by the imaging process. The specimen can be sent subsequently for conventional histopathology examination (typically requiring many hours to days to complete) without risking a compromise in the quality of the specimen. This is not possible with current rapid tissue processing via frozen section because the freezing process damages the tissue, affecting subsequent histological evaluation.

The images of tissue microstructure can be displayed virtually in real-time, while the instrumentation and methodology is fairly simple and inexpensive and can be operated by personnel with no specialized training in this technology. The ability to image specimens without tissue preparation, time-intensive procedures, or prohibitively expensive and complicated instrumentation can enable its acceptance by the medical

community and the health-care system. In addition, because it is a Class I medical device, the Micro-Anatomy Imager does not require FDA approval, thereby speeding its commercial acceptance.

D. List all other applications for which your product can now be used.

The Micro-Anatomy Imager reveals the microstructure of tissue specimens. This capability can enhance three existing technologies:

1. EXPERIMENTAL ANIMAL RESEARCH: There are many cases where this technology could enable the visualization of tissue structure alternations inside an experimental animal due to the formation of a tumor or in response to a chemical or biological agent. Currently, the dynamics of the process are captured by using a large number of experimental animals, which are sacrificed at different times to capture the dynamics of the process. With Micro-Anatomy Imager technology, the dynamics can be captured for each individual animal, thus reducing the number of experimental animal needed to complete the study and potentially obtaining more accurate results. By capturing the drug kinetics and its effects to the body at the cellular level, this approach can find use in research areas such as in cancer and drug discovery research.
2. EDUCATION: In vivo visualization of cell structure and function at the tissue level is currently not possible (or at least very limited) with any existing method. The technology incorporated in the Micro-Anatomy Imager makes tissue visualization possible as well as offering enhanced educational methods over a wide range of skill levels, from the teaching of biology in high school to the training of future medical personnel.
3. GUIDED SURGERY: With proper regulatory approvals, this technology could also be used to obtain in vivo histopathology information inside the human body in real time.

9. TECHNOLOGY DESCRIPTION

A. How does the product or technology operate?

Tissue cells and the space between them contain various biomolecules (such as tryptophan, collagen, elastin, NADH, porphyrins and others, commonly referred to as fluorophores) that fluoresce when exposed to ultraviolet light. These biomolecules are distributed in different concentrations within various cellular compartments, thus naturally “staining” these compartments. As a result, images using the emission of these biomolecules allows for visualization of the cellular compartments.

Imaging of cells in tissues is typically performed either by cutting thin sections of tissues to avoid out-of-focus signals that smear the image or using “optical sectioning” methods that “reject” out-of-focus signals. Typical examples of the latter method are confocal microscopy and nonlinear microscopy. The Micro-Anatomy Imager incorporates a

different imaging method that is based on the principle that using ultraviolet excitation, all generated fluorescence signals are within the focal range of the microscope objective due to the sufficiently short penetration depth of the ultraviolet photons. As a result, this method has optimal signal collection efficiency by utilizing the entire generated signals.

B. What scientific theories, if any, are involved?

The microscopy technology employed in this product is based on two main physical mechanisms:

The first mechanism is associated with the property that ultraviolet light only superficially penetrates tissue (on the order of a few tens of micrometer, depending on tissue type). As a result, the fluorescence signal produced in this superficial tissue layer can be contained within the comparable thickness of the image plane of the microscope, providing high-contrast images with optimized sensitivity without complicated instrumentation and/or signal-intensive methods incorporated in optical sectioning techniques (such as confocal microscopy) that generally causes a large portion of the generated signal (the out-of-focus portion) to be rejected. The mechanism employed by the Micro-Anatomy Imager acquires images based on the emission of all native tissue fluorophores (as they can all be excited with ultraviolet light), and the image quality is independent of the emission wavelength. In addition, images based on the emission of contrast agents can be attained and combined with those of native fluorophores to provide additional molecular (or other types) of information.

The second mechanism is that there is sufficient variability in the concentration of native fluorophores contained within cell compartments. The work performed to date indicates that among the native tissue fluorophores, tryptophan provides the best image contrast in most cases, but other fluorophores also provide important information. This approach enables visualization of the microstructure and organization of the superficial layer of a tissue in a way similar to that provided by conventional staining method in histology (such as Hematoxylin and Eosin staining).

C. What are the building blocks of your technology? Describe the materials, composition, construction, or mechanism of action.

The system is based on the adaptation of conventional fluorescence microscope designs using ultraviolet excitation. The excitation can be obtained by various types of sources, such as lasers and LEDs. The microscope's optics are transparent to near ultraviolet light, so the emission of tissue fluorophores such as tryptophan (which exhibit peak emission at about 330 nanometers) can be detected with optimal sensitivity. Upon collection of the signal by the microscope's optics, different spectral components of the emission can be captured via simultaneous acquisition (using multiple cameras) or

sequentially. The microscope objectives preferably offer long working distance to enable oblique illumination of the sample for enhanced image quality. The camera can be either CCD or CMOS, with preferably a large number of small-size pixels.

Image formation is based on the natural emission of the tissue (autofluorescence) arising from intrinsic tissue fluorophores although the method can work equally efficiently using extrinsic staining fluorescence contrast agents. The microscope can be designed to meet the needs of a wide range of applications, from viewing tissue specimens in a classroom or a laboratory animal facility to imaging excised tissue specimens after surgery, or in endomicroscopy to examine remote locations inside the human body or in experimental animals.

Complete microscopic images of a large specimen is achieved by acquiring multiple images of smaller sections of the sample and stitching them together. The basic images cover an area of about 1 to 2 square millimeters, depending on the choice of microscope objective lenses and detector cameras. Ordinarily, the process of scanning a large specimen very fast can be plagued with motion artifacts. The same is true in using this system for in vivo applications in experimental animals (or in future implementation for in vivo applications in humans) due to vascular and/or muscular movements such as circulation and breathing. The image resolution of a microscope system can be maintained as long as the object being imaged can be kept immobilized during image acquisition. The Micro-Anatomy Imager addresses this problem by using pulsed excitation, in which the entire excitation dose for image acquisition is delivered by a single pulse lasting a few tens of nanoseconds or less. Taking into account that the emission lifetime of the tissue fluorophores is on the order of 10 nanoseconds, the overall timeframe needed for image acquisition can be shorter than approximately 20 nanoseconds. This enables image acquisition much faster than the tissue can move beyond the spatial resolution of the imaging system, which produces images without any inherent motion artifacts.

10. PRODUCT COMPARISON

A. List your product's competitors by manufacturer, brand name, and model number.

There are currently no other competing technologies that offer similar capabilities to the Micro-Anatomy Imager, namely the visualization of tissue cellular structure and organization without the need for tissue preparation (such as application of a contrast enhancing chemical) or use of contrast agents.

Other technologies involve a) confocal microscopy systems and b) nonlinear microscopy systems. Nonlinear microscopy systems require very expensive and complicated laser instrumentation, and the speed of image acquisition is at least two orders of magnitude slower that this technology. Confocal microscopy systems require contrast

enhancement to visualize the cellular compartments, which are delivered either in the form of an application of a substance to increase visibility of the nuclei (such as acetic acid) or the use of a fluorescence contrast agent. In addition, confocal microscopy is still slower that this technology as it only uses a fraction of the generated signal (the in-focus signal) for image acquisition while the entire amount of fluorescence signal produced can be used for image acquisition with this method.

B. Supply a matrix or table showing how the key features of your product compare to existing products or technologies. Use numerical figures to represent performance metrics. Include descriptive comparisons if necessary. For price, and capital and operating costs, use actual dollar amounts or a relative scale ($$, $$$) to show a comparison.

There are no competitive products currently marketed that permit real-time imaging of tissue cells with no required preparation for fast medical diagnosis.

C. Describe how your product improves upon competitive products or technologies.

Compared to current rapid histopathology methods involving staining of frozen sections of a specimen, this method provides improvement in three main areas:

1. A microscopic image of the specimen becomes available for examination in a much shorter period of time.
2. The image is in digital form and can be transmitted via current information technologies that are fully compatible with telemedical applications.
3. The specimen is fully preserved for more detailed analysis at a later time (such as for storing or for conventional histopathological examination)

Compared to other microscopy techniques (confocal and nonlinear), this method provides improvement in three main areas:

1. The scanning speed is faster.
2. The instrumentation is simpler and less expensive
3. No tissue preparation of use of contrast agents is necessary

D. Describe limitations of your product. What criticisms would your competitors offer?

The image resolution of this product is on the order of 500 nanometers. Competitors might raise the issue that this resolution is not sufficient. However, work performed to date by multiple groups using various microscopy methods indicates that resolution on the order of 1 micrometer or slightly higher is sufficient, at least for the vast majority of cases.

11. SUMMARY

Frozen-section assessment of biopsy specimens obtained during surgery is currently a routine part of clinical care. However, this approach is hindered by numerous limitations, including significant processing time required for embedding, freezing, cutting, and staining the specimens as well as the compromised quality of most frozen sections due to this intensive processing. The resulting delays and interpretation challenges limit the use of biopsy or surgical tissue margin evaluation during surgery; as a result, additional surgeries are needed to remove residual cancer present at or near surgical margins.

The need for physical sectioning specimens to thin slices is mitigated with the Micro-Anatomy Imager by introducing a simple and inexpensive optical sectioning method. This approach delivers significant improvements compared to current methods by significantly reducing the need for repeated operations and by providing accelerated patient diagnosis that bypasses current time-consuming procedures. For the first time, visualizing of the structure and function of live cells at the tissue level is readily available. Images are acquired without any need for prior preparation of the tissue or use of contrast agents. In addition, because the microscopic images of the specimens are digitized immediately, their examination by the specialist can be facilitated in the most efficient manner using current information technology tools. Also, by avoiding freezing and cutting small biopsy specimens, the intact specimens will remain available for additional evaluations.

The ability of the system to examine the margins of a removed tissue specimen for residual disease enables detection and removal of the entire tumor during the original surgical procedure. This approach enables an immediate definitive diagnosis, accelerating patient-care decisions and treatments, improving the therapeutic outcome, and reducing patient anxiety and the cost of health care.

The imaging technology incorporated in the Micro-Anatomy Imager can be used with endogenous fluorescent signals arising from native tissue biomolecules, which enables the visualization of the tissue microstructure and organization at the cell level. In addition, it could be used with extrinsic fluorescing contrast agents, which can be absorbed by the tissue specimen following a short exposure to these agents. Such contrast agents can be chosen for their ability to target specific cellular substructures such as the nucleus or the cytoplasm, similar to traditional staining of thin pathology sections. In addition, this technology is compatible with next-generation contrast agents that aim to provide molecular sensitivity to disease detection. Finally, this method is cheaper and more compact than other emerging image-based approaches.

The Micro-Anatomy Imager can be adapted to address research needs to understand the origins and progression of disease, response of the body to bacterial infections, and in drug and contrast agent discovery and development. Furthermore, this instrument

can be used to advance education and has the potential for in vivo diagnosis of disease. Finally, as a Class I medical device, the instrument does not need FDA approval.

In short, the Micro-Anatomy Imager promises to advance disease diagnosis and make health care more efficient and cost-effective, all in a small, cost-effective package. This advance promises to lead to faster intra-operative histopathology consultation, which in turn will reduce the duration of the surgery and prevent repeated surgeries. Consequently, the risk to the patient will be reduced, as well as the costs for the entire healthcare system.

12. AFFIRMATION

By submitting this entry to *R&D Magazine* you affirm that all information submitted as a part of, or supplemental to, this entry is a fair and accurate representation of this product. You affirm that you have read the instructions and entry notes and agree to the rules specified in those sections.

APPENDIX A: DEVELOPMENT TEAM INFORMATION

Team Member Name:	Stavros G. Demos
Title:	Scientist
Organization:	Lawrence Livermore National Laboratory
Address:	7000 East Avenue
City/State:	Livermore, California
Zip/Postal:	94550
Country:	USA
Phone:	925-423-3388
Fax:	925-422-5718
Email:	demos1@llnl.gov

Team Member name:	Shiro Urayama, M.D.
Title:	Professor
Organization:	University of California, Davis
Address:	4150 V Street, PSSB Suite 3500
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA
Phone:	916-734-3759
Fax:	916-734-7908
Email:	surayama@uc davis.edu

Team Member name:	Bevin Lin
Title:	Graduate Student Researcher
Organization:	NSF Center for Biophotonics Science & Technology University of California, Davis
Address:	2700 Stockton Blvd., Suite 1400
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA
Phone:	(916) 734-8600
Fax:	(916) 703-5012
Email:	belin@ucdavis.edu

Team Member name:	Ramez Saroufeem, M.D.
Title:	Associate Professor
Organization:	University of California, Davis
Address:	4400 V Street, PATH Building
City/State:	Sacramento, CA
Zip/Postal:	95817
Country:	USA
Phone:	(916) 734-2529

Fax:	916-734-2560
Email:	ramez.saroufeem@ucdmc.ucdavis.edu

APPENDIX B: MAREKTING AND MEDIA INFORMATION

1. MARKETING

Contact person to handle all arrangements on exhibits, banquet, and publicity.

Name:	Connie Pitcock
Title:	Marketing and Business Development Associate
Organization Name:	Lawrence Livermore National Laboratory
Address:	7000 East Ave., L-795
City/State:	Livermore, CA
Zip/Postal:	94551
Country:	USA
Phone:	925-422-1072
Fax:	925-423-8988
Email:	pitcock1@llnl.gov

2. MEDIA

Contact person for media and editorial inquiries.

Name:	Connie Pitcock
Title:	Marketing and Business Development Associate
Organization Name:	Lawrence Livermore National Laboratory
Address:	7000 East Ave., L-795
City/State:	Livermore, CA
Zip/Postal:	94551
Country:	USA
Phone:	925-422-1072
Fax:	925-423-8988
Email:	pitcock1@llnl.gov